Specification Amendments

Please amend the paragraph bridging pages 3 and 4 as follows:

The present invention provides a method for enhancing the detection of an analyte by amplifying the signal from a binding assay regardless of which detection method is used. This is accomplished by causing the binding reaction to initiate a process that leads to the formation of multiple copies of a product that itself can be detected by any standard sandwich binding assay method. The assay will often be carried out in two stages although it may be possible to combine all reagents at once in some of the homogeneous formats. In the first stage, a sandwich is formed in which one of the receptors of the sandwich has a label and the other ~~second~~ receptor of the sandwich has multiple copies of a substrate associated with it, usually on a support or polymer. Instead of detecting the label directly, the label mediates the oxidative cleavage of a linker attaching the substrate to the product ~~a support~~ and leads to the release of multiple copies of the ~~a~~ product from the support or polymer. The mediation process involves the generation of an oxidant by the label either enzymatically or by irradiation and the subsequent oxidative cleavage of the substrate to release the product. The product can then be detected by a second sandwich binding assay. For this strategy to be successful, at least one of the specific binding reagents required for the sandwich assay must not bind to the substrate either because it is incapable of binding or because the substrate has been removed from the product. Thus, usually at least one binding site must be created in the product that was not present in the substrate. Any sandwich assay method can then be used to detect the product. There are a number of embodiments of the invention that involve substrates that are convertible to a detectable product having two binding sites.

Please amend the paragraph on page 4, lines 20-30, as follows:

In one embodiment of the invention, an oxidant cleavable linker may be used to attach substrate molecules to a detectable product having two binding sites where the substrate is attached to a surface or support. Subsequent to binding of a ~~the~~ target molecule or analyte to produce a sandwich such as described above comprising both the substrate molecules and a ~~the~~

label in close proximity, the label generates an oxidant which cleaves a ~~the~~ linker joining the substrate and the detectable product ~~support~~. The resulting detectable product is released from the surface or support and is physically separated from the substrate by centrifugation, decantation, chromatography or the like. The main advantage of this approach is that any suitable oxidatively cleavable link may suffice. However, this embodiment is usually suitable for heterogeneous assays and the sensitivity of the assay will therefore depend strongly on efficiency of the separation of the free detectable product and detectable product ~~bound receptor~~ to which the substrate is bound.

Please amend the paragraph on page 5, lines 1-14, as follows:

In a second embodiment of the invention, an oxidant cleavable linker may be used to attach a detectable product to ~~a support~~ a substrate molecule where the detectable product comprises ~~having~~ two binding sites wherein one of the binding sites is at least partially masked and is completely unmasked upon cleavage of the link and formation of the product. Masking, whereby a binding site ~~the functional group~~ is unable to bind to its specific binding reagent, can arise simply by virtue of the substrate being bound to a surface. For instance, the substrate may be bound within pores of the support or surface, i.e., an agarose gel, that are too small to accommodate the specific binding reagent. Alternatively, numerous substrate molecules bound to a relatively smooth surface will be unavailable for binding to a specific binding reagent provided that the specific binding reagent is sufficiently bulky, as for example when it is attached to latex particles. Thus release of the substrate with formation of a first binding site ~~the first functional group~~ may be accompanied by unmasking of at least some of a second binding site ~~the second functional group~~. This embodiment of the invention does not require separation of the product from the substrate and is equally suitable for both heterogeneous and homogeneous assays.

Please amend the paragraph on page 5, lines 15-21, as follows:

In a third embodiment of the invention, the substrate attached to a support or surface reacts with the oxidant to simultaneously yield a detectable product comprising two binding sites

~~functional groups~~ that are linked together in a manner that permits binding of two specific binding reagents. For example, two hapten precursors can be attached to the substrate ~~a support or surface~~ by an oxidant cleavable linker and also attached to each other by another chain of atoms that is sufficiently long to allow antibodies to bind to the released haptens once the cleavable link has been severed. This approach would be suitable for homogeneous assays.

Please amend the paragraph bridging pages 5 and 6 as follows:

In a fourth embodiment of the invention, a substrate attached to a support or surface comprises ~~via~~ an oxidant cleavable linker that reacts with an oxidant to release ~~produce~~ a product having a chemically reactive group, usually an electrophilic group. This chemically reactive group is designed to react with a chemical-specific binding reagent, which can be a nucleophile such as an amine or sulfhydryl. The product therefore becomes covalently bound to the specific binding reagent. The product also contains a hapten or ligand that was originally present in the substrate or unmasked as a result of the oxidation or subsequent reaction. For example, oxidation of a substrate that contains one haptenic group linked to a support through an oxidizable linker can yield an active ester as the chemically reactive group. The specific binding reagent could then be an amine, which reacts with the ester. If the amine is attached to a label, reaction with the oxidation product not only releases the product from the support or polymer but also binds the product to the label. If the amine is not attached to a label, it can react with the product to produce a new group, which can serve as a ligand. A labeled receptor for the ligand can then be used in the subsequent detection step. Any assay method that permits detection of a label bound to an antibody can then be used for detection. The fourth strategy likewise allows for homogeneous assays.

Please amend the paragraph on page 9, lines 5-22, as follows:

The present invention provides a method for enhancing the detection of minute quantities of an analyte or target molecule by amplifying the signal from a binding assay that utilizes a catalyst that is capable of generating an oxidant, e.g., a peroxide or singlet oxygen. This catalyst is generally associated with a support or surface such as a particle to form what is referred herein

as a sensitizer particle. The method of the invention entails a first step of forming a sandwich of a first receptor bound to the sensitizer particle, an analyte or target, and a second receptor associated with multiple copies of a substrate. The substrate is attached ~~via oxidant cleavable linker~~ to a support or surface such as a particle to form what is referred to herein as an acceptor particle. The analyte binds to the first and second receptor, drawing the catalyst and substrate in close proximity. When peroxide or singlet oxygen is generated, the oxidant cleavable linker is cleaved, releasing the multiple copies of product that was attached to the substrate by the oxidant cleavable linker ~~substrates as multiple products~~. The product includes two binding sites and may be detected using any standard sandwich assay, which utilizes specific binding reagents to form a detectable ternary complex containing the product. In practicing this invention, it is preferred that one of the specific binding reagents be incapable of binding to substrate when it is bound to the acceptor particle which is required for homogenous assays. In addition, it is preferred that the catalyst generate singlet oxygen as the oxidant and that linkers that attach the substrate to the surface or support be singlet oxygen cleavable.

Please amend the paragraph on page 38, lines 15-25, as follows:

Another aspect of the present invention allows for the reversible coupling of oligonucleotides onto a support or surface via a thioether linker that is cleavable by singlet oxygen. By reacting oligonucleotides labeled with sulfhydryl groups with a surface labeled with alpha iodoacetamide, the oligonucleotides may be attached to the support in high coupling efficiencies and yields. The thioether linkage is stable even on heating the bound surface to 95^0C or exposing the bound surface to irradiation. Exposing the surface to singlet oxygen releases the oligonucleotides from ~~form~~ the surface or support by selective cleavage of the thioether linkage. In practicing the invention, singlet oxygen can be generated by a variety of sources including an enzyme reaction or activation of photosensitizer, preferably a photosensitizer. The bound oligonucleotide may be removed from the surface by singlet oxygen cleavage of the linker.